

**REMARKS**

This is a full and complete response to the Office action dated April 13, 2006.

All comments and remarks of record are herein incorporated by reference. Applicant respectfully traverses the rejections and all comments made in the Office action. Nevertheless, in an effort to expedite prosecution, Applicant provides the following remarks regarding the cited references.

**DISPOSITION OF CLAIMS**

Claims 1-11, and 16-17 are pending in the Application. Applicant has amended claim 1 with support in the application found on page 2-3, paragraphs 7-8, page 10-11, paragraph 40-41, page 12-13, paragraph 48. Claims 2-11 have been amended for clarification. Claims 16-17 have been amended with support found on page 8, paragraph 19. No new matter is added.

**REMARKS REGARDING 35 USC §112**

Claims 1-15 stand rejected under 35 USC §112, second paragraph, as being indefinite. The Applicant respectfully traverses this rejection.

The Examiner alleges that “quantifying a presence” is indefinite because it is unclear whether it is intended to mean detecting the presence or determining a quantity of microorganisms. The Applicant has clarified claim 1 to now recite “assessing a relative quantity” and “utilizing an estimation model to determine the concentration of viable microorganism.” As the claim is clear that a concentration is determined, Applicant respectfully asserts that one of ordinary skill in the art would understand such claim, and therefore requests that the above rejection be withdrawn.

REMARKS REGARDING 35 USC §102

Claims 1-3, 8, 10, 12, and 14 stand rejected under 35 USC §102(b) as being anticipated by **Thomas** (Applied and Environmental Microbiology). Applicant respectfully traverses this rejection.

The Examiner asserts that the instant claims are anticipated because **Thomas** allegedly teaches a method wherein samples are cultured, followed by DNA extraction and species specific detection of organisms after culturing. The Examiner further alleged that a sample in **Thomas** can be quantified based on the strength of a band on electrophoresis gel. Specifically a darker band means more *Listeria monocytogenes* is present, a lighter band means less *Listeria monocytogenes* is present, and no band means that no *Listeria monocytogenes* is present. The Examiner interpreted “quantifying” as determining if a sample is present or absent based on the presence or absence of a specific band on a gel.

Applicant has clarified that the claims recite the use of liquid samples rather than a plating method for determining concentration of the viable microorganism of interest.

Applicant respectfully asserts that **Thomas** does not disclose the presently amended claims. In particular, **Thomas** does not disclose determining a concentration of viable microorganism of interest on a food product based on results of a PCR analysis of liquid samples as is recited in the amended claims.

**Thomas** is directed to a method for detection of *Listeria monocytogenesis* in milk and ground beef samples. **Thomas** indicates this is accomplished by culturing the milk and ground-beef samples in a *Listeria* enrichment broth (LEB), plating the samples with *Listeria* plating medium (LPM), extracting the DNA and then detecting the plated species by polymerase Chain Reaction (PCR). See **Thomas**, abstract.

Applicant wishes to note that because LEB and LPM are utilized, primarily *Listeria* monogenesis will be present after culturing milk and ground-beef samples. After culturing the samples, DNA is extracted by first removing bacteria from LPM plates by use of a dry cotton swab. See **Thomas**, page 2577, column 1, 2<sup>nd</sup> full paragraph. After application of a lysis buffer and other procedures for DNA extraction, it is the sample from the cotton swab which is subject to PCR assays.

Applicant respectfully submits that when LPM plating is used, due to the specificity of the medium, primarily only isolated *Listeria* colonies will be present. The colonies on the plate are not counted, but instead only a cotton swab is used to remove some of the bacteria from the plate. A swab of such a plate will provide varying numbers of bacteria for PCR thereafter, depending on how much bacteria is caught up on the swab. Therefore, the amount of target DNA available for PCR amplification would be dependent on the size of the colony sampled (via cotton swab), the amount of colony taken, and the efficiency of the DNA extraction and NOT the concentration of *Listeria* microorganisms in the original milk or ground beef sample.

By using the plating and swab method as indicated in **Thomas**, no concentration of viable *Listeria* in the original samples of milk or ground beef is determined. Applicant respectfully asserts that the process of **Thomas** merely indicates whether or not the *Listeria* microorganisms were present in the original sample. However, the instant claimed invention enables determining the concentration of a target organism on a food product, not merely whether an organism is present. Therefore, Applicant respectfully submits that **Thomas** does not anticipate the instant claims.

As indicated above, the Examiner also contends that a sample in **Thomas** can be quantified based on the strength of the band in the gel. Applicant respectfully disagrees.

**Thomas** does not teach or suggest a method where band intensity can be used to quantify an organism. As can demonstrated in Figure 3, the band in lanes B and C are similar in size, however, as indicated by Thomas, B is in fact 10 fold greater than that of the band in lane C. Therefore, band intensity does not vary in proportion to the amount of *Listeria* DNA such that one can quantify the organism.

Furthermore, in Fig. 3b, **Thomas** demonstrates that with the amount of identical target DNA, the band intensity can differ dramatically based on the presence of varying amounts of herring sperm. Fig. 3B indicates that increasing the amount of herring sperm DNA decreases band intensity. In this example, band intensity or band strength of a PCR product was inhibited by the presence of DNA that was not target DNA, indicating that band strength is not an indicator of the amount of target DNA.

Additionally, Applicant respectfully asserts that **Thomas** does not disclose preparing a series of progressively dilute test samples by combining portions of the liquid suspension sample with a dilution liquid, incubating the liquid test samples, and conducting PCR. As discussed above, **Thomas** utilizes a plating method, and does not use progressively dilute liquid samples as recited in the instant claims.

Applicant also submits that by use of LPM and LEB, **Thomas** reduces the extent of background organisms because each are designed for the specific growth of *Listeria*. Thus, according to **Thomas**, if a different organism is targeted, a new broth or medium must be made in order to reduce the amount of non-target organisms. The preparation of such specific broths or plating can be expensive and time consuming. The use of plating instead of a liquid suspension can cause complications in the identification and quantification of microorganisms, because many organisms appear similar when cultured on traditional media, and may have a concentration much higher than the target microorganism. See Application, page 2, paragraph 4. The serial dilutions of the instant claimed invention allow for determining concentration even where the target organism is significantly outnumbered by organisms which are not of interest.

Therefore Applicant respectfully submits that **Thomas** does not teach or suggest any means for determining concentration of viable microorganism of interest nor the use of an estimation model to do so. Accordingly, Applicant respectfully requests that the 35 USC §102(b) rejection be withdrawn with regard to claim 1 and its dependent claims.

REMARKS REGARDING 35 USC §103REMARKS REGARDING **THOMAS** IN VIEW OF **PAHUSKI**

Claim 4-6 stand rejected under 35 USC §103(a) as being unpatentable over **Thomas** in view of **Pahuski et al.**, US 5,587,286, ("**Pahuski**"). Applicant respectfully traverses this rejection.

The Examiner alleges that **Pahuski** teaches a method for detecting the amount of organisms in milk. In particular, the Examiner alleges that column 15 of **Pahuski** discloses a test sample divided into 11 different test tubes, which are then plated and allowed to grow over night. The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time of the invention was made to have modified the method of **Thomas** so as to have divided the test sample into multiple portions and cultured each portion for the added benefit of having multiple samples to test which can be used to further confirm the results.

Applicant re-asserts the arguments above with respect to **Thomas**, and further submit that even in view of **Pahuski**, **Thomas** does not teach or suggest the claimed invention.

In column 15, lines 7-34, **Pahuski** indicates that a pasteurized milk sample were added to 11 inoculated milk samples, and then plated in agar and incubated. Applicant respectfully submits that such does not disclose preparing a series of progressively dilute test samples, incubating such samples, and thereafter conducting PCR analysis as recited in the instant claims. As **Pahuski** does not progressively dilute and incubate the 11 inoculated samples, the reference does not disclose or suggest the claimed invention. Instead **Pahuski** uses a plating and colony counting method for determining an amount of bacteria in the 11 samples.

As discussed above, the plating method is not viable where the concentration of non-target microorganisms are in significantly higher concentration than the microorganism of interest. The milk used in **Pahuski** had been pasteurized, and therefore did not have a significant background of other organisms. However, the instant claimed

invention enables determining concentration even in the presence of a significantly higher concentration of organisms which are not of interest.

Furthermore, no estimation model was used to determine the concentration of the microorganism of interest present on a food product based on results of a PCR analysis. According to column 15 in **Pahuski**, the amount of bacteria in the tubes was determined by use of colony counting after plating.

As **Thomas** in view of **Pahuski** does not teach or suggest the claimed limitations, Applicant respectfully requests the rejection be withdrawn.

REMARKS REGARDING **THOMAS** IN VIEW OF **LUCCHINI**

Claims 7, 9, and 15 stand rejected under 35 USC §103(a) as being unpatenable over **Thomas** in view of Lucchini et al. (Federation of European Microbiological Societies) ("**Lucchini**"). Applicant respectfully traverses this rejection.

The Examiner alleges that **Lucchini** teaches a method wherein labeled oligonucleotide probe is hybridized with a sample population in order to quantitate the number of microorganisms in the sample population. The Examiner also alleges that multiplex PCR was performed wherein genus-specific and species specific primers were utilized.

Applicant respectfully asserts that **Thomas** in view of **Lucchini** does not disclose or suggest claims 7 and 9, which depend from claim 1. As noted above, claim 15 has been canceled, and therefore rejection of such claim is no longer applicable.

Applicant re-asserts the arguments above with respect to **Thomas**, and further submit that even in view of **Lucchini**, **Thomas** does not teach or suggest the claimed invention.

**Lucchini** is directed to a method for detecting and identifying a strain of *Lactobacillus gasseri* from animal faeces by means of PCR amplification. In order to measure the population of lactobacilli in mouse faecal samples, **Lucchini** utilizes Rogosa agar medium. See **Lucchini**, page 276, 2<sup>nd</sup> column, 4<sup>th</sup> full paragraph. After plating, multiplex PCR was conducted in **Lucchini** to "type" the colonies grown on the Rogosa

agar medium to determine if the particular strain was present. See Lucchini, page 277, 2<sup>nd</sup> column, 4<sup>th</sup> full paragraph.

As **Lucchini**, as well as **Thomas**, utilize a plating method for determining a count rather than a liquid sample, such references do not disclose the claimed invention. As discussed in detail above, the plating method is not useful where the non-target microorganism has a far higher background concentration than the target microorganism. On a plate, due to the high number of non-target microorganisms, counting of the microorganism of interest would be difficult if not impossible.

Therefore neither **Thomas** or **Lucchini** disclose or suggest preparation of a series of progressively dilute test samples and incubating such samples. Nor do the cited references disclose or suggest conducting a PCR analysis of such dilute liquid samples and utilizing an estimation model to determine concentration from the PCR analysis. Therefore, the Applicant respectfully asserts that the cited references do not disclose or suggest the limitations of dependent claims 7 and 9.

#### REMARKS REGARDING THOMAS IN VIEW OF WANG

Claims 11 and 13 stand rejected under 35 USC §103(A) as being unpatentable over **Thomas** in view of **Wang**. Applicant respectfully traverses this rejection.

The Examiner alleges that **Wang** teaches a method for PCR detection and quantitation of microorganisms found in human and animal fecal samples. The Examiner alleges that quantitation was accomplished by diluting samples down, running the samples on a gel, and comparing the band strength to various controls. The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of **Thomas** so as to quantify the amount of microorganisms in the sample using the method of **Wang**.

Applicant re-asserts the arguments above with respect to **Thomas**, and further submit that even in view of **Wang**, **Thomas** does not teach or suggest the claimed invention.

**Wang** is directed to the specific detection of bacteria in human and animal fecal samples. See Wang, abstract. **Wang** employs PCR titers of various bacterium, wherein the titer is defined as the maximum dilutions for positive PCR results. See Wang, page 1244, 2<sup>nd</sup> column. However, Wang indicates that the PCR method is conducted in situ, as opposed to culture methods which detect bacteria after enrichment. See Wang, page 1245, 2<sup>nd</sup> column, 2<sup>nd</sup> full paragraph. **Wang** indicates that when conducted in situ, PCR analysis detects dead bacteria. See id. Dead bacteria are detected because PCR does not distinguish between DNA obtained from a living organism and DNA obtained from a dead or non-viable organism. See Application, page 14, paragraph 40.

Contrary to **Wang**, however, the instant claims recite an incubation step, wherein after preparing a series of progressively dilute test samples, the samples are then incubated. Such an incubation step causes amplification of the living organism in the samples. As a result, the PCR analysis according to the current claims would detect the presence of viable organisms, whereas dead bacteria or non-viable bacteria would have negligible effect. See Application, page 14, paragraph 40.

As **Wang** does not disclose the incubation step, and furthermore teaches away from such a step, the Applicant respectfully asserts that no prima facie case of obviousness has been established.

Moreover, the PCR titer method of **Wang** is conducted with purified individual bacterial populations and makes no attempt to determine the possible interference non-target DNA will have upon quantification determinations. Consequently, the method of Wang severely underestimates bacterial concentrations. Wang estimated that *Bacterioides* populations were on average  $1 \times 10^5$  or lower. See Wang, fig. 3. However, the data presented by **Wang** is in direct conflict with *Bacteroides* concentrations known in the art, which are usually  $1 \times 10^9$  or higher in humans, as indicated on page 2581 (table 7 and table 8) by **Tannock et al.**, *Analysis of Fecal Microflora of Human Subjects Consuming a Probiotic Product Containing Lactobacillus rhamnosus DR20*, pgs. 2578-2588, Vol 66, NO. 6, 2000, ("**Tannock**") which is enclosed with this reply.

**Wang** also has the drawback that the method sensitivity is organism dependent. For example, the probiotic strain *Bifidobacterium adolescentis* detection limit was 10,000 cells. See Wang, page 1245, 2<sup>nd</sup> column, 2<sup>nd</sup> full paragraph.

Data obtained by direct plating of *Bifidobacteria* or FISH indicate that total *Bifobacterium* via direct bacterial counts performed with selective plating media were  $>1 \times 10^9$ . This data demonstrates the significant drawbacks of the art proposed by **Wang**, which underestimated *Bifidobacterial* species by over 10,000,000 and *Bacteroides* by greater than 10,000 fold respectively.

Additionally, with respect to claim 11, **Wang** does not disclose the use of the Most Probable Number (MPN) method for determining target microorganism concentration. This is a specific method for determining concentration. See Application, page 17, paragraph 48; pages 22-23, paragraphs 61-63. As indicated in the Application on pages 22-23, paragraphs 61-63, after conducting serial dilutions of test samples, incubating the test samples, PCR is conducted to identify positive and negative indications in the test tube of target microorganism(s). After such positives and negatives are determined, a most probable number of the target microorganism can be determined for each dilution in the series from a MPN table, and the most probable number having the best confidence limits can be selected as the most probable number of the target microorganism. See Application, pages 22-23, paragraph 62. With reference back to the known quantity of food product, the concentration of the viable microorganism of interest present on the food product can be determined. See Application, pages 22-23, paragraph 62.

Therefore as the MPN estimation model is not disclosed or suggested by neither **Thomas** or **Wang**, Applicant respectfully requests that the 35 USC §103 rejection be withdrawn.

REMARKS REGARDING STATUTORY DOUBLE PATENTING

Claims 1-15 stand provisionally rejected under 35 USC §101 as claiming the same invention as that of claims 1, 7-20 of copending application No. 10/711,155. Applicant respectfully traverses this rejection.

Applicant respectfully asserts that the copending application, due to the recently filed amendment, now recite a viable microorganism of interest that has “previously been applied to a food product in the course of microbiotically treating the food product.” Therefore, as the instant claims recite a microorganism of interest present in or on a food product, the claims of each copending application are not coextensive in scope.

REMARKS REGARDING NON-STATUTORY DOUBLE PATENTING

Claims 1-15 stand provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2-6 and 21-36 of copending application No. 10/711,155.

Applicant provides herewith a terminal disclaimer thereby obviating the above rejection. Favorable action is solicited.

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**Conclusion**

Having addressed all issues set out in the Office action, Applicant respectfully submits that the claims are in condition for allowance and respectfully request that the claims be allowed.

Respectfully submitted,  
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